Mating system and population genetic structure of the bulldog ant Myrmecia pavida

Article (Published Version)

Myrmecological News	18	25-32	Vienna, March 2013

Mating system and population genetic structure of the bulldog ant *Myrmecia pavida* (Hymenoptera: Formicidae)

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Abstract

Understanding the evolution of the alternative mating strategies of monandry and polyandry is a fundamental problem in evolutionary biology because of the cost-benefit trade-offs associated with mating for females. The problem is particularly intriguing in the social insects because queens in most species appear to be obligately monandrous (i.e., only a single male fathers their offspring), while those in a minority of species have evolved high, and sometimes extreme, polyandry. One group which may shed particular insight is the ant subfamily Myrmeciinae (*Myrmecia* and *Nothomyrmecia*). Here we examine the population and colony genetic structure of the bulldog ant *Myrmecia pavida* CLARK, 1951 by genotyping offspring workers from 45 colonies. We find little evidence of geographic structuring or inbreeding in the population, indicating that the species outbreeds, most probably in mating swarms. We also find that queens of *M. pavida* show moderately high polyandry, with 84% having mated with between two and seven males, and an overall mean observed mating frequency of 3.8. This is significantly higher than previously reported for queens of *Nothomyrmecia macrops*, in which most females mate singly. This was similar to that of *M. pyriformis*, *M. brevinoda*, and *M. pilosula*, the three congenerics for which mating frequencies have recently been reported. The two genera in the Myrmeciinae therefore appear to show multiple transitions in mating frequency and further investigation of the subfamily may be highly informative for disentangling the forces driving the evolution of alternative mating strategies.

Key words: Polyandry, monandry, social insect, mating frequency, paternity.

Myrmecol. News 18: 25-32 (online 4 December 2012)
ISSN 1994-4136 (print), ISSN 1997-3500 (online)

Received 13 January 2012; revision received 7 July 2012; accepted 12 July 2012
Subject Editor: Helge Schlüns

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Introduction

Understanding the selective forces that shape different mating strategies is a fundamental problem that is essential for understanding the evolutionary interplay between natural and sexual selection, as well as speciation. The occurrence of monandry and polyandry in different taxa remains a conundrum in evolutionary biology. Monandry (females each being fertilised by only a single male) is relatively rare and females of most animal species are polyandrous (females each being fertilised by multiple males; ARNQVIST & NILSSON 2000, JENNIONS & PETRIE 2000, SIMMONS 2001, ZEH & ZEH 2003). Polyandry involves greater exposure to the costs of mating, such as sexually transmitted diseases, vulnerability to predators, energetic expenditure and risk of direct harm by males (CHAPMAN & al. 1995, MAKLAKOV & al. 2005, BAER & al. 2006, MCNAMARA & al. 2008). However, polyandry can also provide significant benefits to females. These may include direct material benefits, such as increased sperm, nuptial gifts or paternal care, or indirect genetic benefits, such as diluting genetically incompatible matings or increasing offspring genetic diversity as a bet-hedge against an unpredictable environment (ARNQVIST & NILSSON 2000, JENNIONS & PETRIE 2000, SIMMONS 2005). The co-occurrence of direct and indirect benefits in many animals makes it hard to disentangle their relative importance. The fact that the costs of polyandry will also vary between taxa makes it additionally difficult to determine whether greater benefits or reduced costs are driving the evolution of higher mating frequencies in specific taxa. The occurrence of polyandry is in addition of particular interest because it results in significant potential for postcopulatory sexual selection, as well as for evolutionary conflicts within and between the sexes (BAER 2011).

The eusocial Hymenoptera (all ants, some bees and some wasps) have proved useful models for understanding the evolution of mating strategies. Polyandry reduces the indirect fitness benefits which select for social behaviour through kin selection (HAMILTON 1964, BOOMSMA 2007). Accordingly, and in contrast to most animals, the reproductive females (queens) in most eusocial insects are exclusively monandrous, with polyandry being a derived state (HUGHES & al. 2008a). The costs of polyandry in most species are also likely to be relatively high, because the mating period is one of the riskiest parts of a queen's life as she is not protected by workers (CROZIER & FJERDINGSTAD 2001, SUMNER & al. 2004, BAER & al. 2006). Nevertheless, approximately a third of social Hymenoptera have evolved

facultative, low levels of polyandry (queens being fertilised occasionally by two or three males) and nine clades have evolved higher levels of polyandry (> 90% of queens being inseminated by multiple males, with the number of males also often being higher; honeybees, *Vespula* yellowjacket wasps, army ants, leaf-cutting ants, *Pogonomyrmex* harvester ants, *Cataglyphis* desert ants, *Pachycondyla* ponerine ants, and *Plagiolepis* and *Cardiocondyla* ants; HUGHES & al. 2008a, HUGHES & al. 2008b). Polyandry has almost certainly not evolved in these taxa for direct, material benefits because males do not provide paternal care or nuptial gifts, do not force females to mate multiply, and multiple mating does not result in females storing significantly greater quantities of sperm (FJERDINGSTAD & BOOMSMA 1998, CROZIER & FJERDINGSTAD 2001, SCHLÜNS & al. 2005). However, empirical studies with social insects have produced some of the strongest evidence for females instead gaining genetic benefits from polyandry, including by diluting genetically incompatible matings and increasing offspring genetic diversity (BAER & SCHMID-HEMPEL 1999, COLE & WIERNASZ 1999, TARPY & PAGE 2002, CAHAN & KELLER 2003, HUGHES & BOOMSMA 2004, JONES & al. 2004, HUGHES & BOOMSMA 2006, GOODISMAN & al. 2007, MATTILA & SEELEY 2007, SEELEY & TARPY 2007, SCHWANDER & KELLER 2008, WADDINGTION & al. 2010, CONSTANT & al. 2012).

One group of social insects which may be particularly informative for understanding the evolution of alternative mating strategies is the Australasian ant subfamily Myrmeciinae. It currently contains 90 described species of the infamous *Myrmecia* bulldog ants that are classified into nine species groups and are conspicuous components of most Australian ecosystems, as well as the "living fossil" ant *Nothomyrmecia macrops*, which is the only extant member of its genus (TAYLOR 1978, OGATA & TAYLOR 1991, BOLTON & al. 2007). The subfamily is located in a key, understudied region of the ant phylogeny and is basal to the vast majority of ants for which mating strategies are known (BRADY & al. 2006, MOREAU & al. 2006, RABELING & al. 2008). They are regarded as one of the "primitive" ant subfamilies, having retained many ancestral characters, such as relatively small colonies and little queen-worker dimorphism, but also include some species with more derived traits, such as simple forms of worker polymorphism and larger colonies (GRAY 1974, CRAIG & CROZIER 1979, HÖLLDOBLER & TAYLOR 1983, CROSLAND & al. 1988, HIGASHI & PEETERS 1990, PEETERS 1997, DIETEMANN & al. 2002). Here we investigate the genetic structure of *Myrmecia pavida* CLARK, 1951, a member of the *M. gulosa* species group, from south-western Australia. We establish the number of queens in colonies and their mating frequencies using microsatellite markers. We also examine the population genetics of *M. pavida*, with a sample of 46 colonies of three morphs, collected from seven sites.

Materials and methods

Sample collection and identification

Between 12 and 71 individuals were collected in 2009 from each of 46 *Myrmecia* nests located at seven sites near Perth, South Western Australia, with the furthest sites being 37 km apart (Fig. S1 and Tab. S1, Appendix, as digital supplementary material to this article at the journal's web pages). After the nest entrance was located, individuals entering or departing the nest were collected and stored immediately in 100% ethanol. Three morphs were identified based on colouration patterns that differed clearly and consistently between nests, with workers within a nest always belonging to the same morph. Morph 1 was found mainly to the west of Perth (28 out of 29 nests), while the only nest of morph 2 and most nests of morph 3 (15 out of 16 nests) were collected to the east of Perth. All specimens were identified by Dr. Steve Shattuck of the Entomology Division of the Australian National Insect Collection (CSIRO) as belonging to the species *Myrmecia pavida* (Hymenoptera, Formicidae, Myrmeciinae).

DNA sequences for the *28S rRNA* gene (*28S*, 622 bp) and the *Long Wave Opsin* gene (*OP*, 1640 bp) from two randomly selected individuals of each of the morphs were produced using primers and protocols as in HASEGAWA & CROZIER (2006) and compared with published sequences for *Myrmecia* species (GenBank accession numbers AB208449 to AB208482 and AB207106 to AB207135). As the sequences from individuals belonging to each morph were identical, and considering the characteristics of the genes used (HASEGAWA & CROZIER 2006), no further individuals were sequenced. A phylogenetic reconstruction using Maximum Parsimony (bootstrap consensus tree, 1000 replicates) was produced using the combined data (28S + OP = 2262 bp) for each morph, plus 19 other *Myrmecia* species for which the sequences of the two genes were available and *Nothomyrmecia macrops* as the outgroup. Pairwise genetic distances (number of base substitutions per site) among species belonging to the *M. gulosa* group were calculated in MEGA 4 (TAMURA & al. 2007) using the Maximum Composite Likelihood method in order to assess if the three morphs could be considered different taxa.

Genotyping

Microsatellite loci developed for *Nothomyrmecia macrops* (see SANETRA & CROZIER 2000) and *Platythyrea punctata* (see SCHILDER & al. 1999) were tested for amplification and polymorphism in *M. pavida*. Only the primers designed for *N. macrops* were found to successfully amplify homologous loci in *M. pavida*. Five of these loci were found to be polymorphic in *M. pavida* (*Nmac1, Nmac115, Nmac23, Nmac53*, and *Nmac18*) and were used to genotype workers from all of the colonies sampled. Reactions were carried out in 15 µl final volume with 165 µM dNTPs, 1.5 mM $MgCl_2$, 0.4 µM of each primer, 0.4 units of Taq and 2 µl of template DNA extracted from legs using 200 µl of a 5% Chelex® solution. The thermocycling profile was 94°C for two minutes, followed by 35 cycles at 93°C for 30 seconds, annealing temperature for 30 seconds and 72°C for 30 seconds, with a final extension at 72°C for ten minutes. The annealing temperature for *Nmac18* was 53.5°C, for *Nmac1* and *Nmac23* was 51°C and for *Nmac53* and *Nmac115* was 52°C. Twelve to 32 samples (Tab. S1, Appendix) from each nest were amplified using labelled primers and the sequences analysed with a 3130*xl* Genetic Analyzer and GeneMapper Software 3.0 (ABI).

Population genetic structure

The microsatellite data were used to calculate population pairwise genetic distances (in the form of transformed pairwise F_{ST} values between nests) (SLATKIN 1995) and mole-

cular diversity indices. Population structure was analysed using hierarchical analyses of molecular variance (AMOVA) as implemented in Arlequin 3.5 (EXCOFFIER & LISCHER 2010), where covariance components of the variance of gene frequencies, due to inter-individual and inter-population differences, are used to compute fixation indexes or F statistics (WRIGHT 1965). Two-level AMOVAS were carried out to test for possible genetic structure of all individuals sampled, with separate analyses being run using worker genotypes with colonies grouped by morph, collection site or as a single population, or using the inferred genotypes of the parent queens or males in order to verify if dispersal of reproductives could be affecting the genetic structure of the population. The relationship between genetic distance (pairwise F_{ST} values) and geographic distance was also examined using Mantel tests for the three data sets (workers, queens and males). All analyses were carried out using Arlequin 3.5.

Colony genetic structure and mating frequencies

The genotypes of the mother queens and their mates in each colony were inferred using the program COLONY v2.0 (JONES & WANG 2009). The analyses were run in triplicate using different random seeds to check for convergence of the results. Allelic drop out was set to 0, error rates set to 0.01, and the allelic frequencies for the population were inputted and allowed to be updated during runs. All simulations were run allowing for polygamy of both males and queens, and the highest likelihood combination of -gyny and -andry identified. Males may contribute unequally to offspring and so the effective mating frequency (k_{eff}) was calculated to control for paternity skew (PAMILO 1993). The effective mating frequencies correcting for sample size (k_{e3}) was also calculated (NIELSEN & al. 2003), as this is more reliable at high mating frequencies or low sample sizes. Pedigree relatedness (g) among workers of the same matriline was estimated using the effective paternity frequency as $g = 0.25 + 1 / (2k_{est})$ (CROZIER 1970). Paternity skew (B) was calculated using the program SKEW CALCULATOR 2003 (Peter Nonacs; http:www.obee.ucla.edu/Faculty/Nonacs). The observed mating frequencies, effective mating frequencies and paternity skew were compared between the morphs and collections sites using Kruskal-Wallis tests. These variables were also compared between *M. pavida* and the published data for four other myrmeciine species, again using Kruskal-Wallis tests, with Dunn tests for all pairwise comparisons. The proportions of queens that were inseminated by more than one male were compared between the species using a Fisher's exact test, with Fisher's exact tests also carried out for each pairwise comparison.

Results

Phylogenetic analysis

The phylogenetic reconstruction based on the *28S* and *OP* genes placed the three morphs of *M. pavida* into a monophyletic clade together with the sequences from all the members of the *M. gulosa* species group (Fig. 1). Morphs 1 and 3 were most closely related, with morph 2 being placed as their sister group in the MP tree. Genetic distances among the three morphs were at least one order of magnitude lower than those among other species pairs from the *M.*

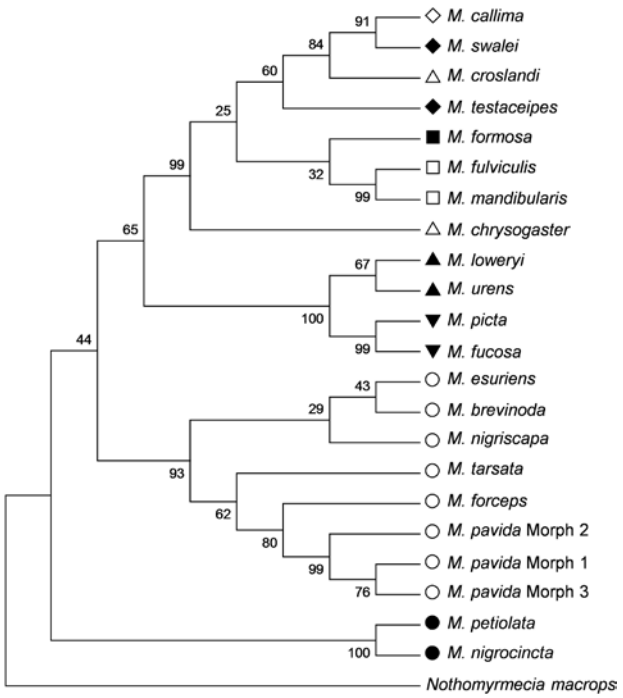

Fig. 1: Phylogeny reconstruction of the relations among 22 *Myrmecia* taxa, including the three morphs identified during the study. Strict consensus MP tree based on partial sequences of the 28S rRNA and Opsin gene (2262 bp). Numbers in nodes represent bootstrap probabilities (1000 replicates). ● *M. nigrocincta* group; □ *mandibularis* group; ■ *M. aberrans* group; ▲ *M. urens* group; ○ *M. gulosa* group; ◆ *M. tepperi* group; ◇ *M. cephalotes* group; Δ *M. pilosula* group; ▼ *M. picta* group.

gulosa group (Tab. 1), indicating that the three morphs are likely to belong to the same species.

Genetic diversity and population structure

The five polymorphic microsatellite loci used had from six (*Nmac115*) to 42 (*Nmac53*) alleles, with observed overall heterozygosity values from 0.3 to 0.9 and average gene diversity from 0.531 ± 0.338 to 0.630 ± 0.376 (Tab. S2, Appendix). There was no significant correlation across colonies between the pairwise F_{ST} values and the geographical distance between nests, regardless of whether the analysis used the worker genotypes, or the inferred genotypes of the parent queens and males ($r^2 < 0.005$ and $P > 0.05$ in all cases based on 1023 permutations). The AMOVAs showed significant effects of structure, but the proportion of variation explained was extremely low, regardless of whether the analysis was based worker genotypes grouped by morph, collection site or as a single population, or were based on the inferred genotypes of the parent queens or males (Tab. S3, Appendix).

Colony genetic structure and mating frequencies

For 39 of the 46 nests, the COLONY analysis found the colonies to be most likely monogynous. For a further four nests, the worker genotypes could not be explained by a single mother queen due to a single anomalous worker. Excluding these workers raised the cluster probabilities of their colonies from < 0.3 to > 0.95, so the individual workers were excluded from further analysis. For the remain-

Tab. 1: Estimates of Evolutionary Divergence (number of substitutions per site, below diagonal) between concatenated partial sequences (2174 nt) of the 28S rRNA and Opsin gene from eight *Myrmecia* taxa belonging to the *M. gulosa* group. Taxa one to five have species status, taxa six to eight represent the three morphs found in this study. Standard error estimates (above diagonal) obtained by bootstrap (1000 replicates). Estimates were produced in MEGA 4 using a Maximum Composite Likelihood method, considering variation among sites (gamma = 1).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1. M. nigriscapa	–	0.005	0.005	0.005	0.005	0.005	0.004	0.005
2. *M. esuriens*	0.02	–	0.005	0.005	0.004	0.004	0.004	0.004
3. *M. forceps*	0.02	0.019	–	0.005	0.004	0.003	0.003	0.003
4. M. brevinoda	0.02	0.019	0.023	–	0.004	0.005	0.005	0.005
5. *M. tarsata*	0.018	0.018	0.014	0.018	–	0.003	0.003	0.003
6. Morph 1	0.017	0.016	0.011	0.02	0.012	–	0.001	0.001
7. Morph 2	0.017	0.015	0.01	0.019	0.012	**0.001**	–	0.001
8. Morph 3	0.017	0.016	0.011	0.02	0.012	**0.001**	**0.001**	–

ing three nests, the COLONY analysis indicated they were most likely polygynous (Colonies 37 and 45, each with two queens, and Colony 35 with five queens). No evidence of exchange of workers between nests located at the same collection site was observed.

The observed mating frequencies of queens from nests of morph 1 and morph 3 varied from one to seven mates, while the single queen of morph 2 had singly mated (Fig. 2). There was no significant difference between morphs in either observed or effective mating frequency (χ^2 = 3.53, P = 0.171 and χ^2 = 4.21, P = 0.122 respectively, df = 2, N = 53). There was also no significant effect of the site from which the ants were collected (χ^2 = 11.9, P = 0.101 and χ^2 = 13.1, P = 0.071 for observed and effective mating frequency respectively, df = 7, N = 53). Overall, 13% of the *M. pavida* queens had been inseminated by a single male, 26% by two males, 30% by three males, while 23% and 6% of queens had mated with four and five males respectively (Fig. 2). In the colonies where multiple matings were observed, male contributions varied considerably (Fig. 3), with the majority male siring between 93% and 22% of the offspring. Paternity skew (B) at the population level was 0.051 and was significant in ten of the colonies after controlling the false discovery rate (Fig. 2b). The overall effec-

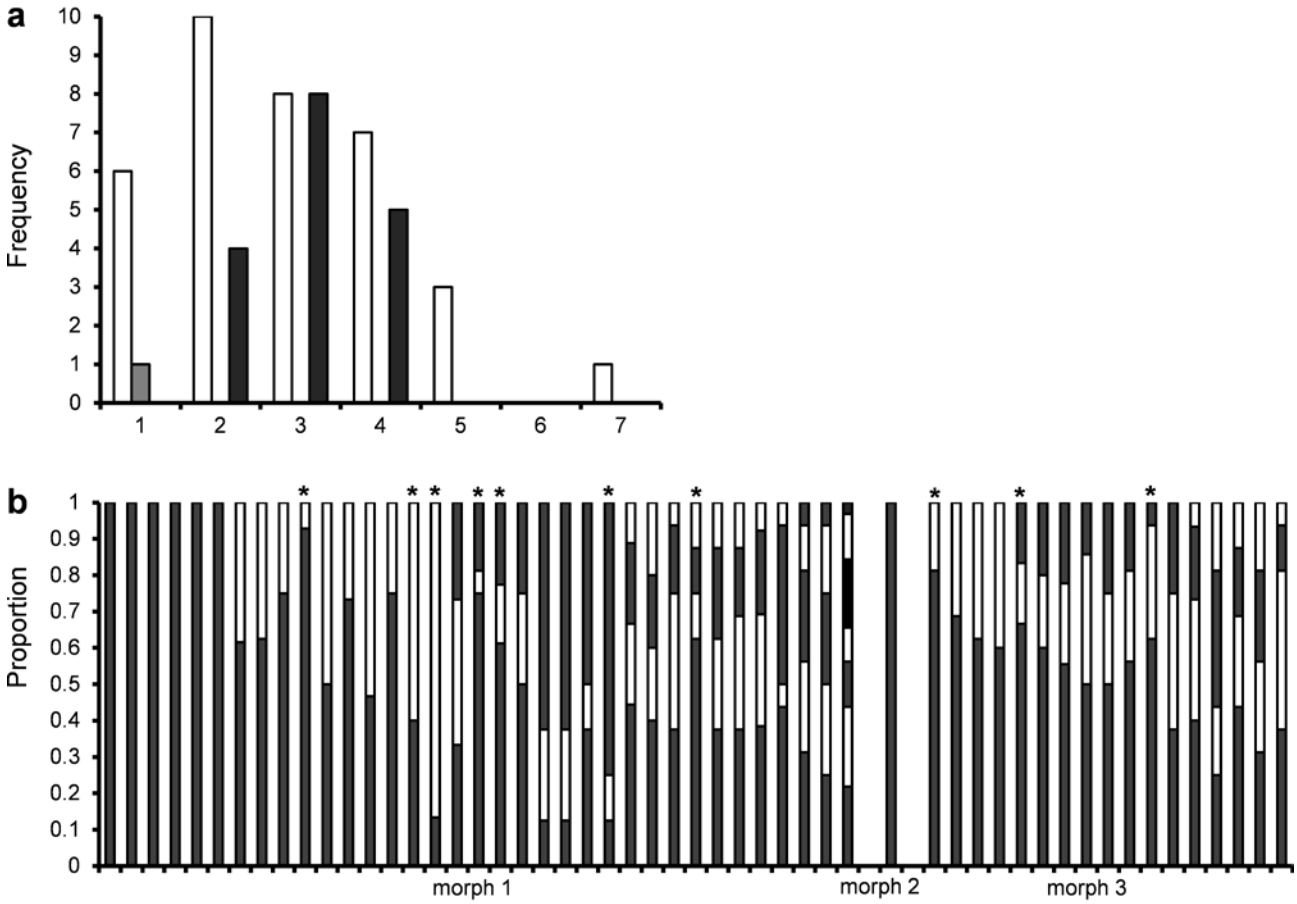

Fig. 2: Mating frequencies of 45 *Myrmecia pavida* colonies studied. a) Frequency distribution of number of matings (*k* observed) of the queens for morph 1 (white bars, 28 queens), morph 2 (grey bars, one queen) and morph 3 (black bars, 16 queens). b) Paternity skew for the mates of each queen, with each column representing the proportions of the different patrilines found among the offspring of the queen. Asterisks above columns indicate significant paternity skew after controlling the false discovery rate.

Tab. 2: Estimates of paternity and relatedness within *Myrmecia pavida* nests. Values indicate the average (± standard deviation) worker sample size (n), observed (k_o), effective (k_{eff}) and effective when correcting for sample size (k_{e3}) number of males that had inseminated a queen, with its associated variance (V), and relatedness (g) among nestmates.

	# queens	n	k_o	K_{eff}	K_{e3}	V	g
Morph 1	34	14.3 ± 6.34	2.9 ± 1.3	2.8 ± 1.6	3.0 ± 1.7	0.2 ± 0.3	0.5 ± 0.1
Morph 2	1	16	1	1	1	0	0.75
Morph 3	17	15.8 ± 1.09	3.1 ± 0.8	2.9 ± 0.1	3.1 ± 0.8	0.2 ± 0.1	0.4 ± 0.1
Overall	52	14.8 ± 5.2	2.9 ± 1.2	2.8 ± 1.4	3.0 ± 1.5	0.2 ± 0.3	0.5 ± 0.1

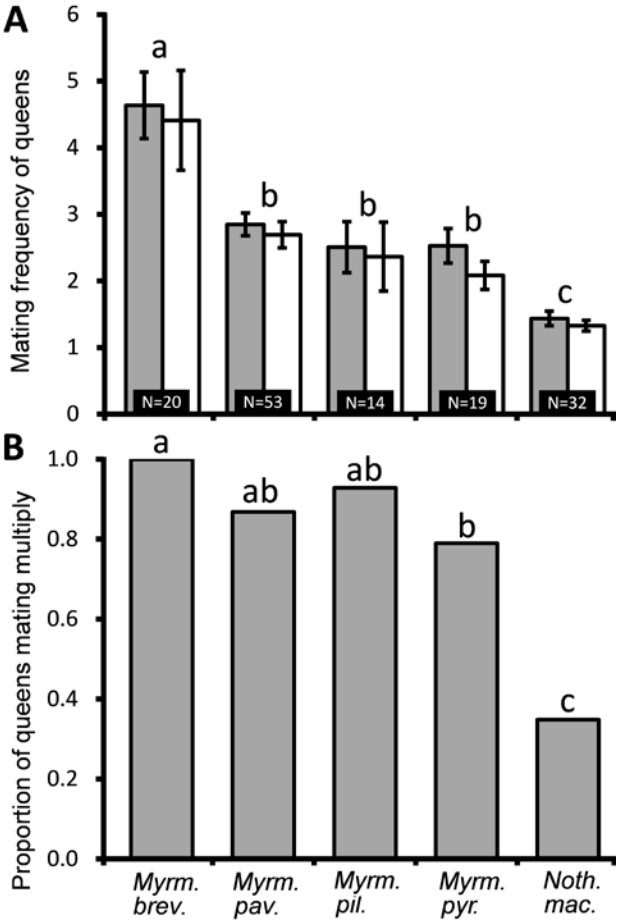

Fig. 3: A) Mean ± standard error, observed (grey columns) and effective (white columns) mating frequencies and B) proportions of queens mating multiply for the five Myrmeciinae species that have been studied: *Myrmecia brevinoda* (see QIAN & al. 2011), *M. pavida* (this study), *M. pilosula* (see QIAN & al. 2012), *M. pyriformis* (see SANETRA 2011), and *Nothomyrmecia macrops* (see SANETRA & CROZIER 2001). The numbers of queens sampled for each species are indicated at the base of the columns in (A). Different letters above the columns indicate species which differed significantly from one another in pairwise tests (species differed similarly for observed and effective mating frequencies).

tive mating frequency for the 53 queens was 3.0 ± 1.5 (Tab. 2). Across all colonies, the average relatedness of nestmate workers was 0.48 ± 0.11 (min: 0.11, max: 0.75, mode: 0.75, median: 0.46).

The five species of Myrmeciinae for which mating frequencies are now known had similar paternity skew (χ^2 = 5.19, N = 106, df = 4, P = 0.269), but differed significantly from one another for both observed and effective mating frequencies (respectively χ^2 = 48.7, P < 0.001, χ^2 = 42.8, P < 0.001, N = 138, df = 4). *Nothomyrmecia macrops* queens were inseminated by significantly fewer males than any of the *Myrmecia* species, and *M. brevinoda* queens were inseminated by significantly more males than queens of the other *Myrmecia* species (Fig. 3A). The species also differed significantly in the proportions of queens mating with multiple males (P < 0.001). Significantly fewer *N. macrops* queens mated multiply than did queens of any of the *Myrmecia* species, and the proportion was also significantly lower in *M. pyriformis* than in *M. brevinoda* (Fig. 3B).

Discussion

Colonies of the bulldog ant *Myrmecia pavida* were generally found to be headed by single, polyandrous queens, with 39 out of 46 nests being monogynous, and 46 out of 53 queens having been inseminated by multiple males. Most queens had mated with two to four males, but some had mated with up to seven males. The mating frequencies were similar to other *Myrmecia* species, but significantly higher than *Nothomyrmecia macrops*. The phylogenetic analysis confirmed all three morphs studied are most likely the same species, *Myrmecia pavida*. Although there was a significant relationship between the genetic and geographic distance between samples, location explained only a very small proportion of the genetic variation in the samples and there was no evidence of inbreeding, indicating that the mating system involves outbreeding by dispersing males and / or females.

Although the three morphs of *M. pavida* sampled differed markedly and consistently in colouration, the phylogenetic reconstruction indicated that they all belong to the same species within the *M. gulosa* group. Cryptic species are common in ants and have been reported previously in *Myrmecia* (CROZIER & al. 1995, SEIFERT 2009), but the genetic distance between the morphs was an order of magnitude less than that to other species in the *M. gulosa* group. Morph 1 was found predominantly at the north-eastern sites and morph 3 at the south-western sites, so their differences in colouration may relate to microclimatic variation.

Genetic variation among nests within sites was much higher than that between sites in all of the analyses, and geographic distance explained only a very small amount of the total genetic variation. Males and females have different dispersal rates in *Nothomyrmecia macrops* (see SANETRA & CROZIER 2003), but there was no evidence for this being the case in *M. pavida*, and the nests appear to belong to a single panmictic population. Queens in some ant species, such as army ants and *Proformica* ants (FERNANDEZ-

ESCUDERO & al. 2002, KRONAUER & al. 2007), mate within or close to the nest. However, the lack of genetic structure in the population indicates that this is not the case in *M. pavida*. At least five other *Myrmecia* species, all from the *M. gulosa* group, have been observed to engage in mating and dispersal flights (CLARK 1951, CROSLAND & al. 1988, QIAN & al. 2011), and the genetic data here suggests this is also true of *M. pavida*.

The frequency of polyandry recorded here for *M. pavida* is relatively high compared to other eusocial insects, most of which are obligately monandrous (HUGHES & al. 2008a, HUGHES & al. 2008b). The average effective mating frequency of 3.0 was moderate, making the level of polyandry in *M. pavida* lower than that seen in *Acromyrmex* leaf-cutting ants (SUMNER & al. 2004, NEHRING & al. 2011, CONSTANT & al. 2012), *Pogonomyrmex* harvester ants (VOLNY & GORDON 2002, GADAU & al. 2003, WIERNASZ & al. 2004, POL & al. 2008), army ants (KRONAUER & al. 2007) or honey bees (TARPY & al. 2004), but comparable to that in *Vespula* wasps (GOODISMAN & al. 2007), *Atta* leaf-cutting ants (EVISON & HUGHES 2011), *Pachycondyla* (see KELLNER & al. 2007), *Plagiolepis* (see THURIN & al. 2011), *Cardiocondyla* (see LENOIR & al. 2007) and most *Cataglyphis* ants (PEARCY & al. 2004, TIMMERMANS & al. 2008, TIMMERMANS & al. 2010). The moderate level of polyandry seen in *M. pavida* is within the range which can be explained by the various suggested benefits of polyandry (CROZIER & FJERDINGSTAD 2001). Most colonies had only a single reproducing queen (monogyny), and so *M. pavida* fits with the relationship across the eusocial Hymenoptera of species being either generally polyandrous or generally polygynous, but rarely both (KELLER & REEVE 1994, KRONAUER & BOOMSMA 2007, HUGHES & al. 2008b).

Mating frequency estimates are now available for five members of the Myrmeciinae subfamily of ants: *Myrmecia pavida* (this study), two other members of the *M. gulosa* group, *M. brevinoda* and *M. pyriformis* (see QIAN & al. 2011, SANETRA 2011), one member of the *M. pilosula* species group, *M. pilosula* (see QIAN & al. 2012), and *Nothomyrmecia macrops* (see SANETRA & CROZIER 2001), which is the only member of its genus. All four *Myrmecia* species have significantly greater levels of polyandry than *N. macrops*. This difference is most striking in terms of the proportions of queens mating multiply, with only 35% of *N. macrops* queens doing so compared with 79 - 100% of *Myrmecia* queens, but was also the case for both observed and effective mating frequencies. In addition, there was also a smaller, but still significant, difference within *Myrmecia*, with *M. brevinoda* having a higher level of polyandry than the other species. This should be treated with some caution as a different method was used to estimate the mating frequencies for *M. brevinoda* (see SANETRA 2011). However, the difference between *Myrmecia* and *Nothomyrmecia macrops* seems conclusive.

It is notable that *N. macrops* has much smaller colonies (~ 60 workers) than members of the *M. gulosa* species group of *Myrmecia*, which can have several thousand workers (HIGASHI & PEETERS 1990, HÖLLDOBLER & WILSON 1990, OGATA & TAYLOR 1991, SANETRA & CROZIER 2001, SANETRA 2011). It may therefore be that these species of *Myrmecia* have evolved higher levels of polyandry than *N. macrops* due to some benefit associated with having larger colonies. This may relate to improved division of labour (CROZIER & FJERDINGSTAD 2001). Although division of labour in all Myrmeciinae appears to be relatively simple with solitary foraging, it may be more complex in the *M. gulosa*-group species of *Myrmecia* as these exhibit worker polymorphism with workers ranging in size from 13 - 36 mm, whereas workers of *N. macrops* are essentially monomorphic (TAYLOR 1978, HÖLLDOBLER & TAYLOR 1983, HIGASHI & PEETERS 1990, DIETEMANN & al. 2002, DIETEMANN & al. 2004). There are a number of records of *Myrmecia* being infected by entomopathogenic fungi, nematodes, protozoa and parasitoids (SCHMID-HEMPEL 1998, BOOMSMA & al. 2005), and it may also be that benefits relate to improved resistance to disease. There is no reason to believe that the impact of genetically incompatible matings, such as due to sex-locus load, is greater in *Myrmecia* than *N. macrops*, so this seems a less likely explanation for the evolution of greater levels of polyandry in the *Myrmecia* species.

Although the ant subfamily Myrmeciinae contains only 91 described species, with all but one in a single genus, its taxa exhibit colony sizes ranging from several thousand workers to less than ten (HIGASHI & PEETERS 1990, OGATA & TAYLOR 1991, BOLTON & al. 2007), making it ideal for testing the general prediction that polyandry is positively correlated with colony size across social insect species. We have shown here that *Myrmecia* bulldog ant queens show moderate levels of polyandry, are significantly more polyandrous than queens heading the smaller colonies of *Nothomyrmecia macrops*, and that there may be significant variation in mating frequency even between different species of bulldog ants. It seems likely that the *Myrmecia* genus has considerable potential to shed light on the costs and benefits driving the evolution of different mating strategies, and the implications of these for the evolution of sexually selected traits.

Acknowledgements

We are grateful to Steve Shattuck and the CSIRO Insect Identification Service for identifying the ant samples, and the Department of Environment, Water, Heritage and Arts for permission to collect and export the samples. We also thank members of the Hughes Lab and two anonymous reviewers for constructive comments which improved the manuscript, and the British Ecological Society, Linnean Society and Leverhulme Foundation for funding the work.

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